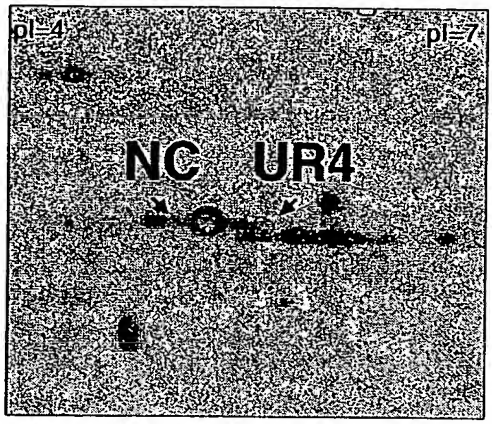
(A) CEM



(B) CEM/VLB

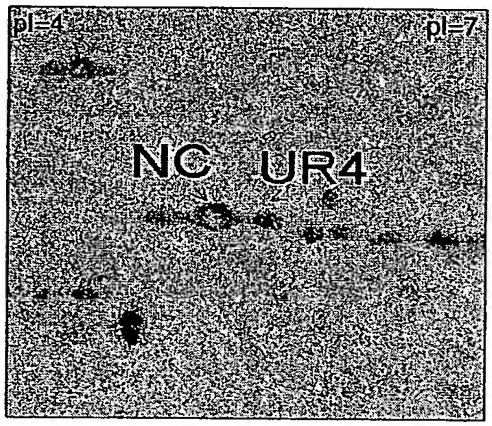


Figure 1

(A) Isoform 2 Results

ProFound - Search Result Summary

Version 4.10.8

Protein Candidates for search 20010608200436-0394-208172123151 [73182 sequences searched]

Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (1)	%	pI	kDa	
+1	1.0e-000	2.36	gi|5729877|ref|NP_006588.1| heat shock 70kD protein 8; heat shock 70kD protein 8 (HSP73); heat shock cognate protein 71 kDa; heat shock 70kd protein 10 (HSC71) [Homo sapiens]	25	5.4	71.11	®

(B) Search Parameters

Details for rank 1 candidate in search 20010608200436-0394-208172123151

gi|5729877|ref|NP_006588.1| heat shock 70kD protein 8; heat shock 70kD protein 8 (HSP73); heat shock cognate protein 71 kDa; heat shock 70kd protein 10 (HSC71) [Homo sapiens]
gi|13639862|ref|XP_006086.2| heat shock 70kD protein 8 [Homo sapiens]
gi|123648|sp|P11142|HS7C_HUMAN HEAT SHOCK COGNATE 71 KDA PROTEIN
gi|87625|pir||A27077 dnaK-type molecular chaperone - human
gi|32467|emb|CAA68445.1| (Y00371) 71 Kd heat shock cognate protein [Homo sapiens]
gi|13273304|gb|AAK17898.1|AF352832_1 (AF352832) constitutive heat shock protein 70 [Homo sapiens]

Sample ID: NO CHANGE [Pass 0]
Measured peptides: 20
Matched peptides: 12
Min. sequence coverage: 25%

(C)

*12 Peptides Sequenced**	Measured Mass(M)	Avg/ Mono	Computed Mass	Error (ppm)	Residues Missed Start	To	Cut	Peptide sequence
-1	1250.623	M	1250.611	10	237	246	0	MVNHFIAEFK (1)+O@M;
-2	1252.592	M	1252.608	-13	302	311	0	FEELNADLFR
-3	1406.696	M	1406.712	-12	237	247	1	MVNHFIAEFKR (1)+O@M;
-4	1479.743	M	1479.746	-2	300	311	1	ARFEELNADLFR
-5	1486.756	M	1486.693	42	37	49	0	TTPSYVAFTDTER
-6	1690.705	M	1690.718	-7	221	236	0	STAGDTHLGGEDFDNR
-7	1786.968	M	1786.982	-8	172	188	1	IINEPTAAAIAYGLDKK
-8	1820.874	M	1820.883	-5	57	72	1	NQVAMNPTNTVFDAKR (1)+O@M;
-9	1837.001	M	1837.005	-2	326	342	1	LDKSQIHDIVLVGGSTR
-10	1951.065	M	1951.052	7	452	469	1	DNNLLGKFELTGIPPAPR
-11	1980.996	M	1980.990	3	138	155	0	TVTNAVVTVPAYFNDSQR
-12	2773.258	M	2773.318	-21	424	447	0	QTQTFTTYSDNQPGVLIQVYEGER

*25% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed.

Figure 2

1 MSKGPAVGIDLGTTYSCVGVFQHGKVEIIANDQGNR**TTPSYVAFTDTER**LIGDAAK**NQVA**

61 **MNPTNTVFDAKR**LIGRRFDDAVVQSDMKHWPFMVVNDAGRPKVQVEYKGETKSFYPEEVS

121 SMVLTKMKEIAEAYLGK**TVTNAVVTVPAYFNDSQR**QATKDAGTIAGLNVLR**IINEPTAAA**

181 **IAYGLDKK**VGAERNVLIFDLGGGTFDVSILTIEDGIFEVK**STAGDTHLGGEDFDNRMVNH**

241 **FIAEFKR**KHKKDISENKRAVRRLRTACERAKRTLSSSTQASIEIDSLYEGIDFYTSIT**RA**

301 **RFEELNADLFR**GTLDPVEKALRDAK**LDKSQIHDIVLVGGSTR**IPKIQKLLQDFFNGKELN

361 KSINPDEAVAYGAAVQAAILSGDKSENVQDLLLLDVTPLSLGIETAGGVMTVLIKRNTTI

421 PTK**QTQTFTTYSDNQPGVLIQVYEGER**AMTK**DNNLLGKFELTGIPPAPR**GVPQIEVTFDI

481 DANGILNVSAVDKSTGKENKITITNDKGRLSKEDIERMVQEAEKYKAEDEKQRDKVSSKN

541 SLESYAFNMKATVEDEKLQGKINDEDKQKILDKCNEIINWLDKNQTAEKEEFEHQQKELE

601 KVCNPIITKLYQSAGGMPGGMPGGFPGGGAPPSGGASSGPTIEEVD

BOLD = Peptides covered by MS MALDI analysis

Figure 3

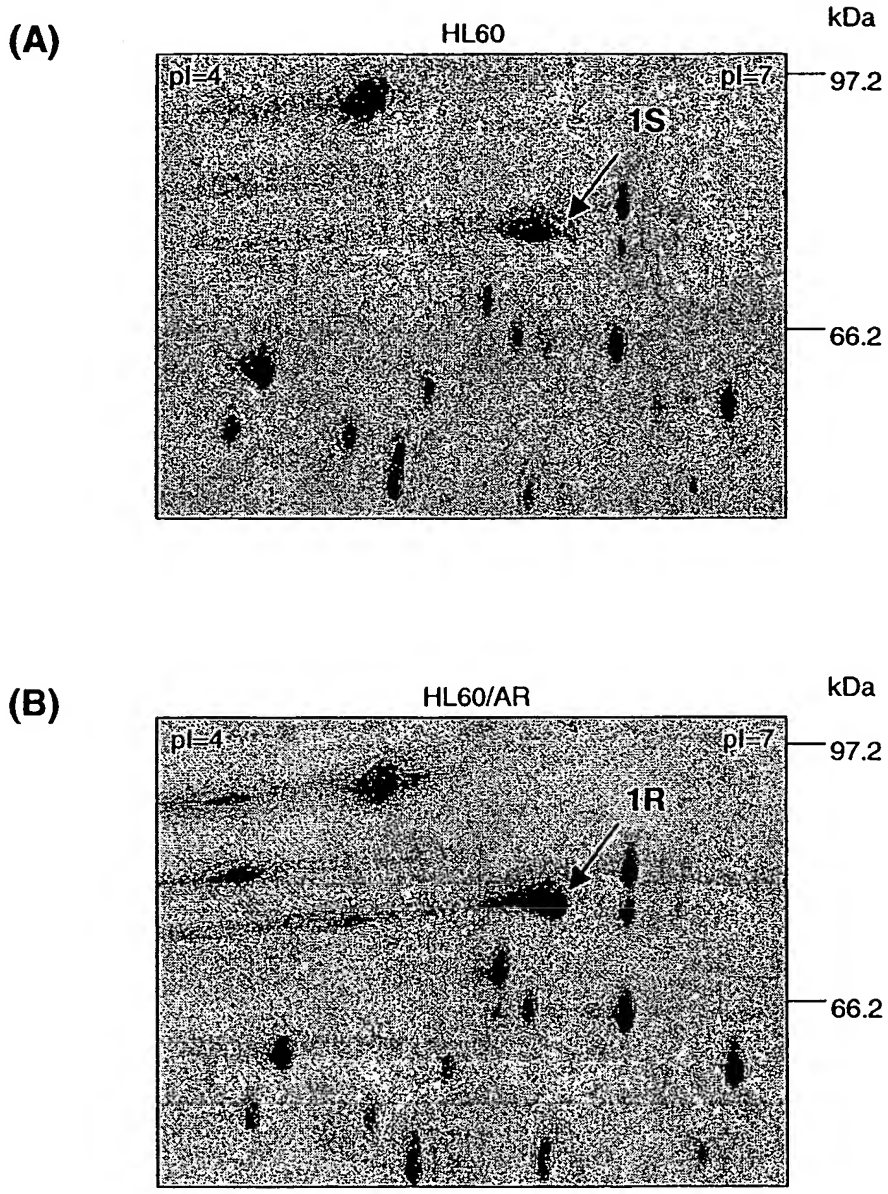


Figure 4

(A)

ProFound - Search Result Summary — Version 4.10.5 — The Rockefeller University Edition

Protein Candidates for search BD8595B9-865C-280FB325 (88967 sequences searched)

Rank	Probability	Est'd Z	Protein Information and Sequence Analysis Tools (T)	%	pI	kDa	
1	1.0e+000	2.43	T gi|5729877|ref|NP_006588.1| (NM_006597) heat shock 70kD protein 8; heat shock 70kD protein 8 (HSP73); heat shock cognate protein; 71 kDa; heat shock 70kd protein 10 (HSC71) [Homo sapiens]	26	5.4	71.11	R

(B) Search Parameters

Input Summary

Date & Time	Mon Dec 17 01:01:33 2001 UTC (Search Time: 2.80 sec)
Sample ID	
Database	NCBInr (2001/12/11)
Taxonomy Category	Homo sapiens (human)
Protein Mass Range	60 - 90 kDa
Protein pI Range	4.0 - 5.5
Search for	Single protein only
Digest Chemistry	Trypsin
Max Missed Cut	2
Modifications	+C2H3ON@C(Complete), +O@M(Partial) C22H37N4O4S@K(Partial)
Charge State	MH+
Peptide Masses (Da,Average)	
Tolerance(AVG)	100.00 ppm
Peptide Masses (Da,Monoisotopic)	1199.667 1253.610 1401.805 1407.785 1463.128 1480.751 1487.698 1691.729 1830.802 1838.043 1966.104 1982.001 1994.000 2231.141 2275.180 2278.081 2757.251 2774.246 2998.417 3548.224
Tolerance(MON)	100.00 ppm
Number of Peptides	20

(C)

*12 Peptides Sequenced**

Measured peptides	20
Matched peptides	12
Min. sequence coverage	26%

Boxed peptides are biotinylated

-1	1198.659	M	1198.666	-6	160	171	0	DAGTIAGLNVLR
-2	1252.602	M	1252.608	-5	302	311	0	FEELNADLFR
-3	1406.717	M	1406.712	46	237	247	0	MVNHFIAEFKR
-4	1479.744	M	1479.746	-2	300	311	1	ARFEELNADLFR
-5	1486.690	M	1486.693	-2	37	49	0	TTPSYVAFTDTER
-6	1690.721	M	1690.716	2	221	236	0	STAGDTHLGGEDFDNR
-7	1837.035	M	1837.005	16	326	342	1	LDKSQIHDIVLVGGSTR
-8	1965.096	M	1965.083	7	112	125	2	GFLDEVEKALFDAK
-9	1980.994	M	1980.989	2	138	155	0	GVTNAVVTVPAYFNDSQR
-10	1980.994	M	1980.956	19	518	533	3	MVQEAEKYKAEDEKQR
-11	2274.172	M	2274.136	16	57	72	1	NQVAMNPTNTVFDAKR
-12	2773.238	M	2773.318	-29	424	447	0	QTQTETTYSDNQPGVLIQVYEGER
-13	2997.409	M	2997.452	-14	540	557	3	NSLESYAFNMKATVEDEK

*26% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed. Note that 2 and 4 represent the same peptide

Figure 5

1 MSKGPAVGIDLGTTYSCVGVFQHGKVEIIANDQGNR**TTPSYVAFTDTER**LIGDAAK***NQVA***

61 ***MNPTNTVFDAKR***LIGRRFDDAVVQSDMKHWPFMVVNDAGRPKVQVEYKGETKSFYPEEVS

121 SMVLTKMKEIAEAYLGK**TVTNAVVTVPAYFNDSQR**QATK**DAGTIAGLNVLR**IINEPTAAA

181 IAYGLDKKVGAERNVLIFDLGGGTFDVSILTIEDGIFEVK**STAGDTHLGGEDFDNRMVNH**

241 **FIAEFKR**KHKKDISENKRAVRRLRTACERAKRTLSSSTQASIEIDSLYEGIDFYTSITR**A**

301 **RFEELNADLFR*GTLDPVEKALRDAK*LDKSQIHDIVLVGGSTR**IPKIQKLLQDFFNGKELN

361 KSINPDEAVAYGAAVQAAILSGDKSENVQDLLLLDVTPLSLGIETAGGVMTVLIKRNTTI

421 PTK**QTQTFTTYSDNQPGVLIQVYEGER**AMTKDNNLLGKFELTGIPPAPRGVPQIEVTFDI

481 DANGILNVSAVDKSTGKENKITITNDKGRLSKEDIER**MVQEAEKYKAEDEKQR**DKVSSK***N***

541 ***SLESYAFNMKATVEDEK***LQGKINDEDKQKILDKCNEIINWLDKNQTAEKEEFEHQQKELE

601 KVCNPIITKLYQSAGGMPGGMPGGFPGGGAPPSGGASSGPTIEEVD

BOLD = Nonbiotinylated peptides specific for HSC70
Underlined & Italics = Biotinylated peptides

Figure 6

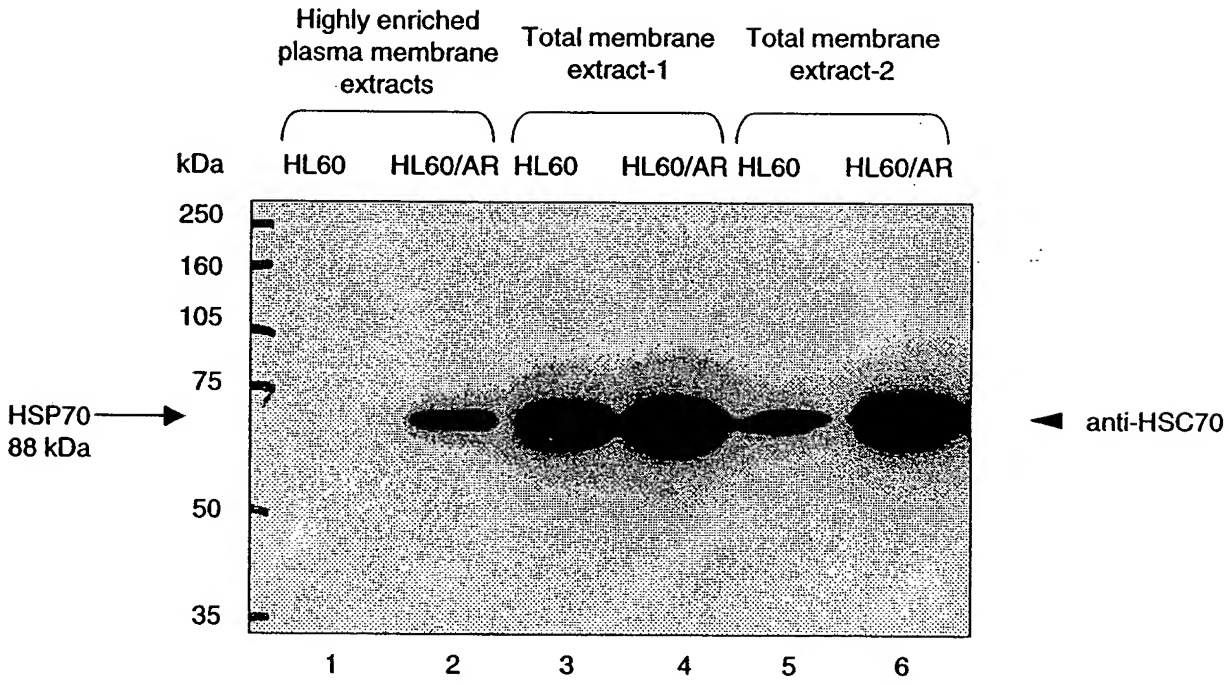


Figure 7

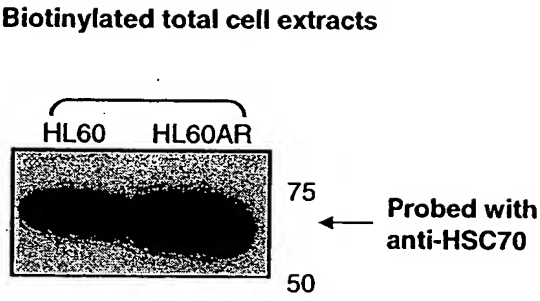


Figure 8A

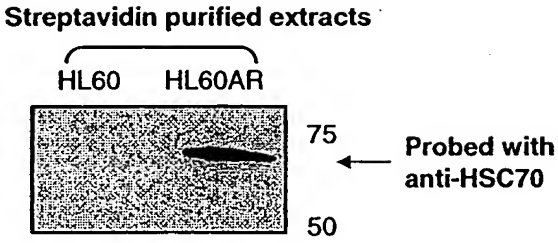


Figure 8B

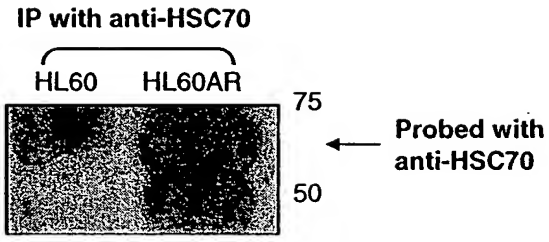


Figure 8C

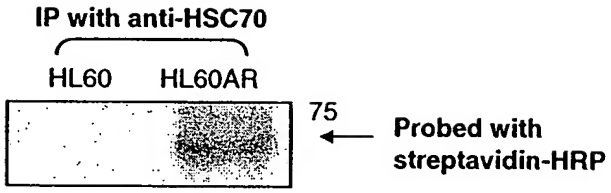


Figure 8D

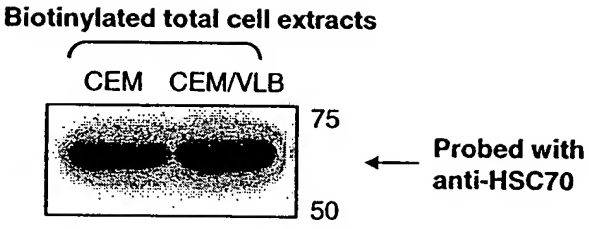


Figure 9A

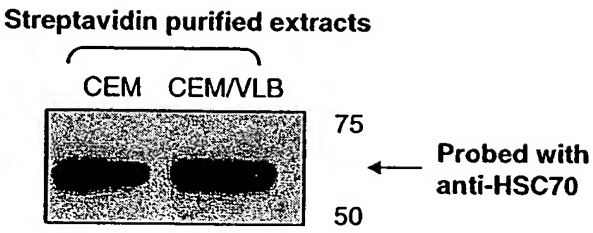


Figure 9B

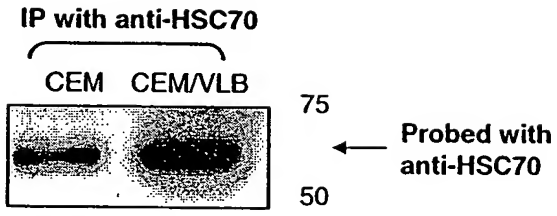


Figure 9C

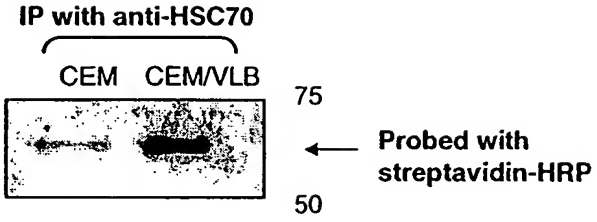


Figure 9D

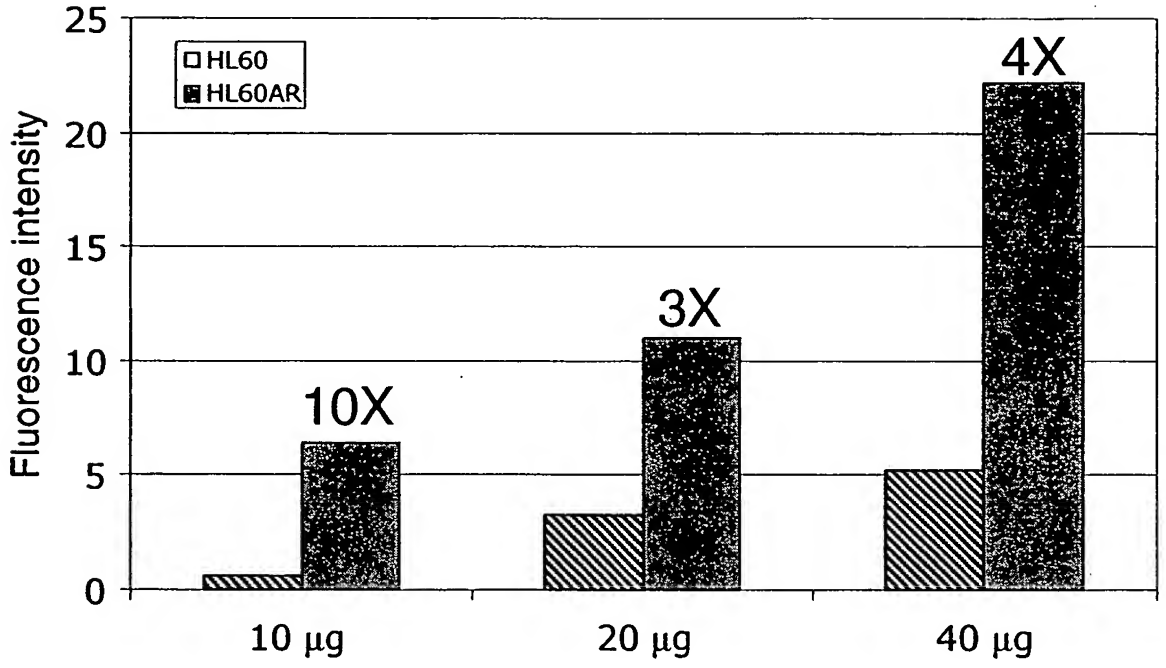


Figure 10A

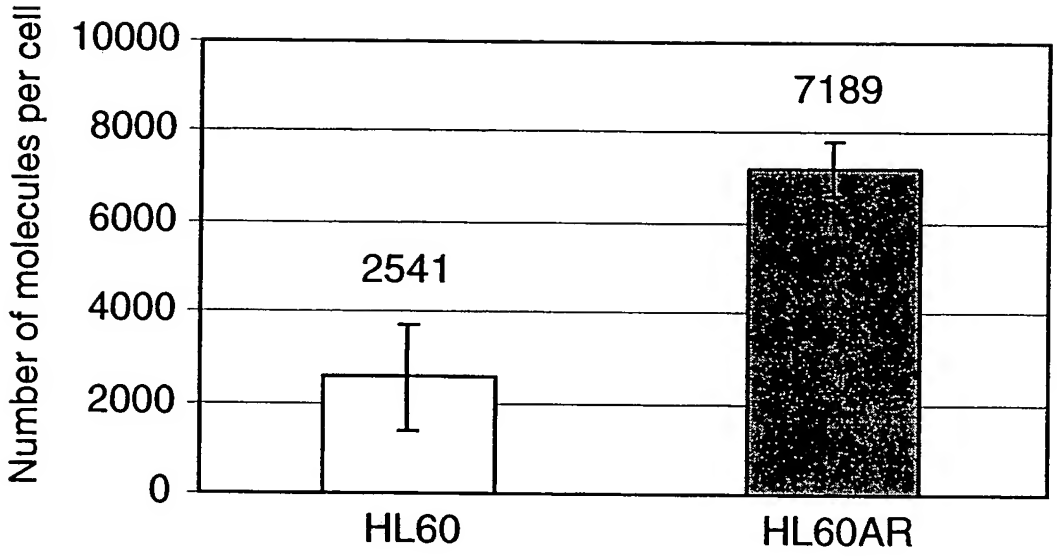


Figure 10B

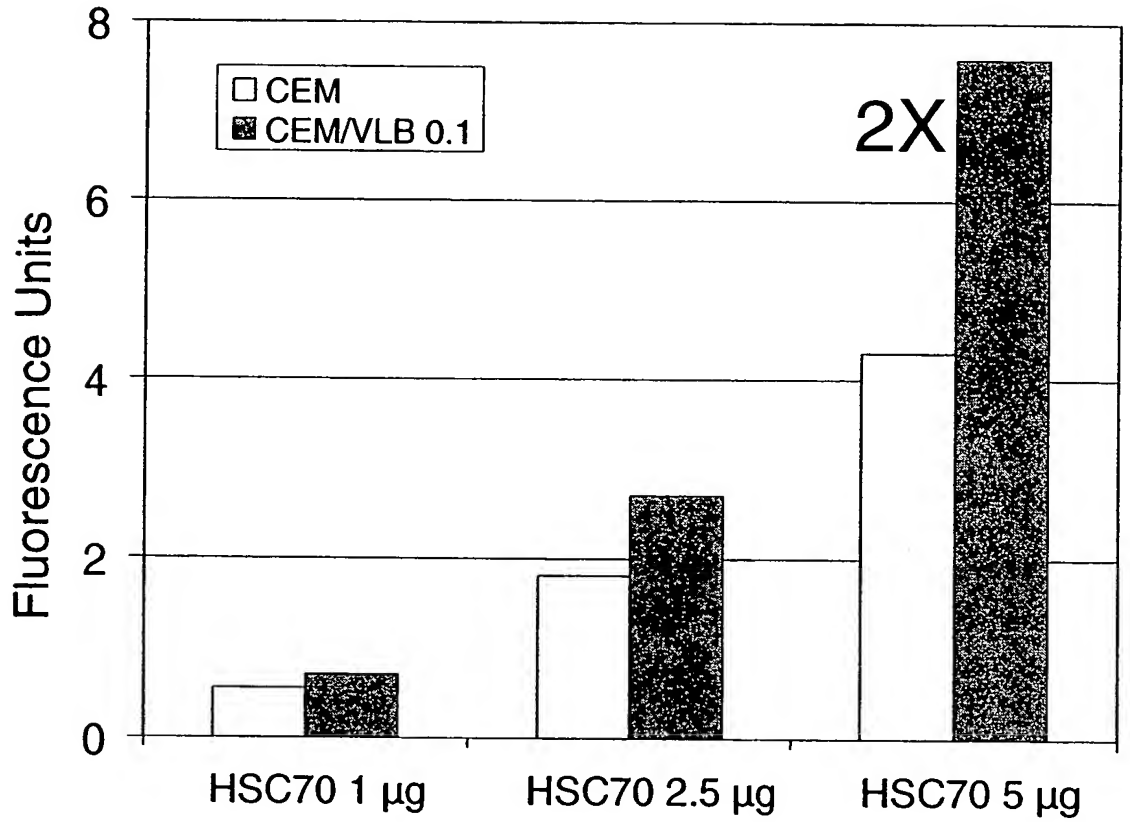


Figure 11A

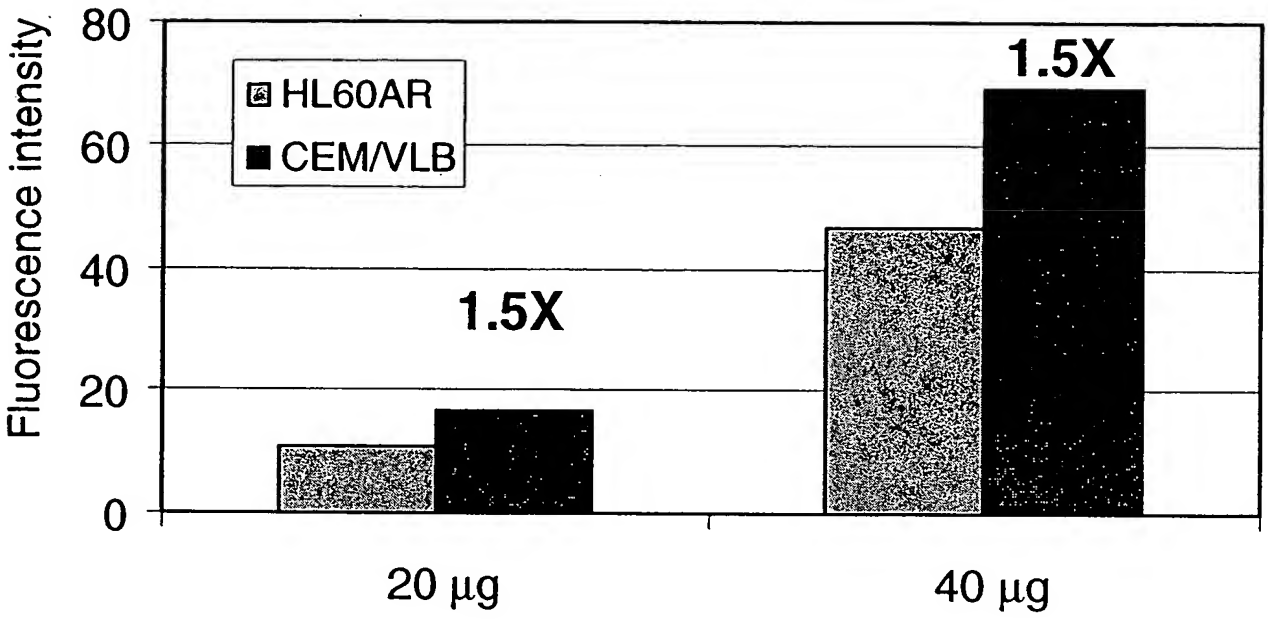


Figure 11B

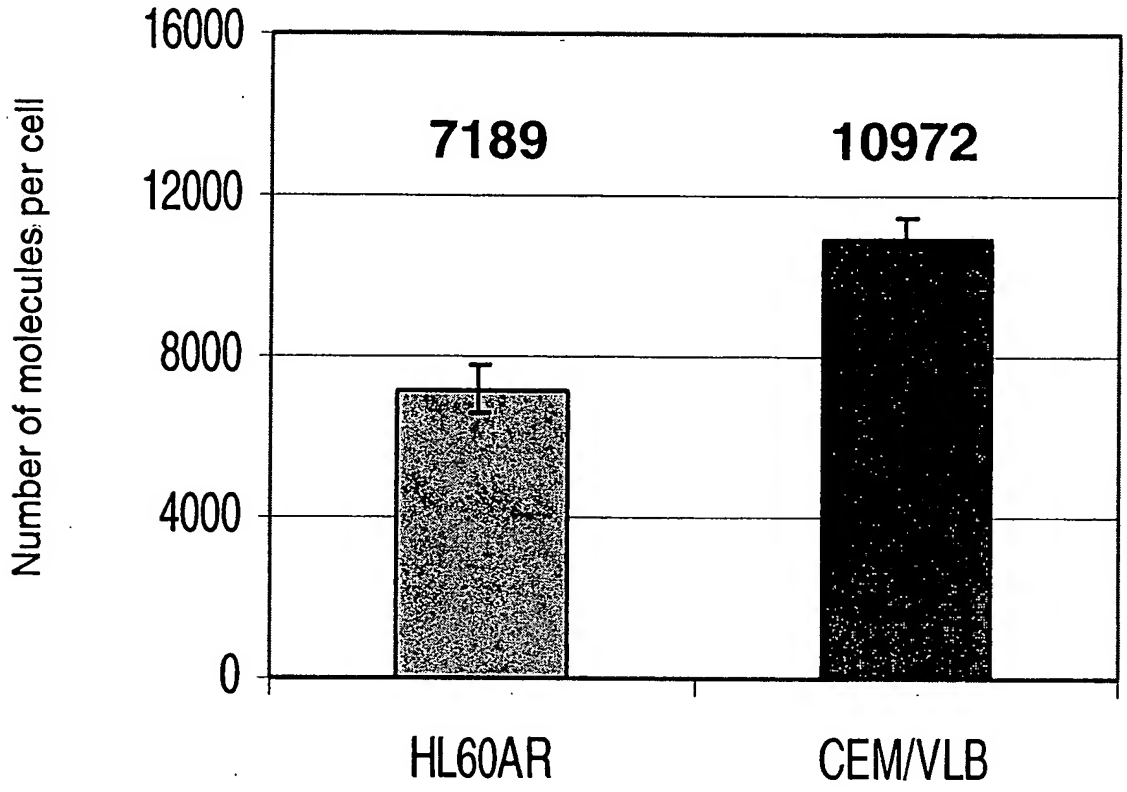


Figure 11C

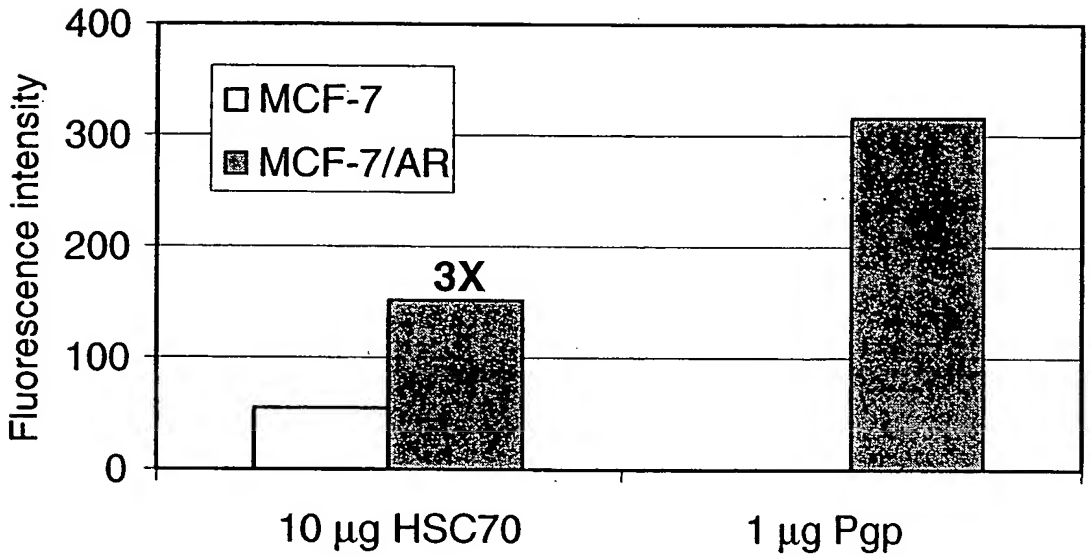


Figure 12A

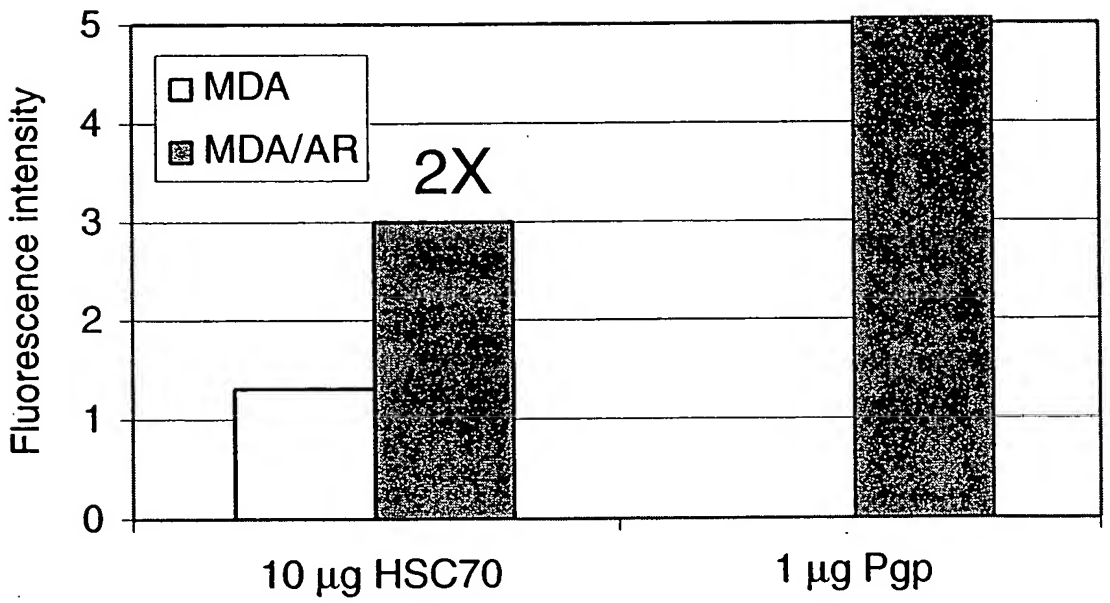


Figure 12B

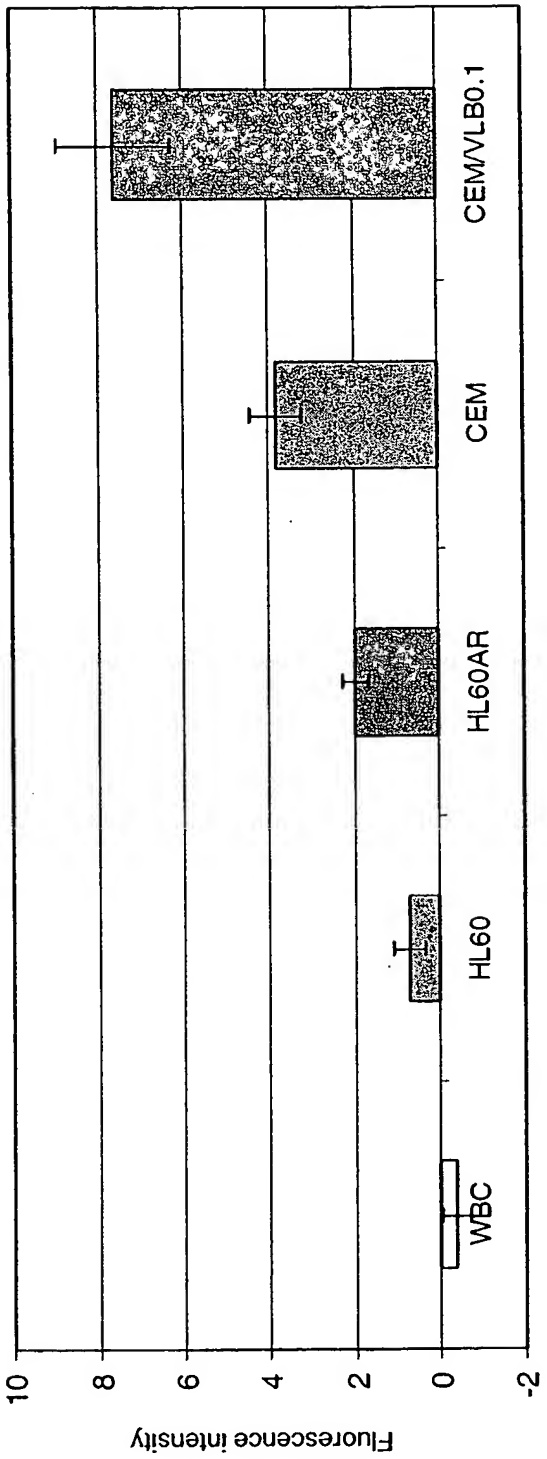


Figure 13A

FIGURE 14

A

POLYPEPTIDE SEQUENCE OF HUMAN HSC70

(GENBANK ACCESSION NO. AAK17898 (SEQ ID NO. 1))

```
  1 MSKGPAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL IGDAAKNQVA
 61 MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVVNDAGR PKVQVEYKGE TKSFYPEEVS
121 SMVLTKMKEI AEAYLGKTVT NAVVTVPAYF NDSQRQATKD AGTIAGLNVL RIINEPTAAA
181 IAYGLDKKVG AERNVLIFDL GGGTFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVNH
241 FIAEFKRKHK KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA
301 RFEELNADLF RGTLDPVEKA LRDAKLDKSQ IHDIVLVGGS TRIPKIQKLL QDFFNGKELN
361 KSINPDEAVA YGAAVQAAIL SGDKSENVQD LLLLDVTPLS LGIETAGGVM TVLIKRNTTI
421 PTKQTQTFTT YSDNQPGVLI QVYEGERAMT KDNNLLGKFE LTGIPPAPRG VPQIEVTFDI
481 DANGILNVSA VDKSTGKENK ITITNDKGRL SKEDIERMVQ EAEKYKAEDE KQRDKVSSKN
541 SLESYAFNMK ATVEDEKLQG KINDEDKQKI LDKCNEIINW LDKNQTAEKE EFEHQQKELE
601 KVCNPIITKL YQSAGGMPGG MPGGFPGGGA PPSGGASSGP TIEEVD
```

B

DNA SEQUENCE OF HUMAN HUMAN HSC70

(GENBANK ACCESSION NO. AF352832 (SEQ ID NO. 2))

```
   1 ATGTCCAAGG GACCTGCAGT TGGTATTGAT CTTGGCACCA CCTACTCTTG TGTGGGTGTT
  61 TTCCAGCACG GAAAAGTCGA GATAATTGCC AATGATCAGG GAAACCGAAC CACTCCAAGC
 121 TATGTCGCCT TTACGGACAC TGAACGGTTG ATCGGTGATG CCGCAAAGAA TCAAGTTGCA
 181 ATGAACCCCA CCAACACAGT TTTTGATGCC AAACGTCTGA TTGGACGCAG ATTTGATGAT
 241 GCTGTTGTCC AGTCTGATAT GAAACATTGG CCCTTTATGG TGGTGAATGA TGCTGGCAGG
 301 CCCAAGGTCC AAGTAGAATA CAAGGGAGAG ACCAAAAGCT TCTATCCAGA GGAGGTGTCT
 361 TCTATGGTTC TGACAAAGAT GAAGGAAATT GCAGAAGCCT ACCTTGGGAA GACTGTTACC
```

421 AATGCTGTGG TCACAGTGCC AGCTTACTTT AATGACTCTC AGCGTCAGGC TACCAAAGAT

481 GCTGGAACTA TTGCTGGTCT CAATGTACTT AGAATTATTA ATGAGCCAAC TGCTGCTGCT

541 ATTGCTTACG GCTTAGACAA AAAGGTTGGA GCAGAAAGAA ACGTGCTCAT CTTTGACCTG

601 GGAGGTGGCA CTTTTGATGT GTCAATCCTC ACTATTGAGG ATGGAATCTT TGAGGTCAAG

661 TCTACAGCTG GAGACACCCA CTTGGGTGGA GAAGATTTTG ACAACCGAAT GGTCAACCAT

721 TTTATTGCTG AGTTTAAGCG CAAGCATAAG AAGGACATCA GTGAGAACAA GAGAGCTGTA

781 AGACGCCTCC GTACTGCTTG TGAACGTGCT AAGCGTACCC TCTCTTCCAG CACCCAGGCC

841 AGTATTGAGA TCGATTCTCT CTATGAAGGA ATCGACTTCT ATACCTCCAT TACCCGTGCC

901 CGATTTGAAG AACTGAATGC TGACCTGTTC CGTGGCACCC TGGACCCAGT AGAGAAAGCC

961 CTTCGAGATG CCAAACTAGA CAAGTCACAG ATTCATGATA TTGTCCTGGT TGGTGGTTCT

1021 ACTCGTATCC CCAAGATTCA GAAGCTTCTC CAAGACTTCT TCAATGGAAA AGAACTGAAT

1081 AAGAGCATCA ACCCTGATGA AGCTGTTGCT TATGGTGCAG CTGTCCAGGC AGCCATCTTG

1141 TCTGGAGACA AGTCTGAGAA TGTTCAAGAT TTGCTGCTCT TGGATGTCAC TCCTCTTTCC

1201 CTTGGTATTG AAACTGCTGG TGGAGTCATG ACTGTCCTCA TCAAGCGTAA TACCACCATT

1261 CCTACCAAGC AGACACAGAC CTTCACTACC TATTCTGACA ACCAGCCTGG TGTGCTTATT

1321 CAGGTTTATG AAGGCGAGCG TGCCATGACA AAGGATAACA ACCTGCTTGG CAAGTTTGAA

1381 CTCACAGGCA TACCTCCTGC ACCCCGAGGT GTTCCTCAGA TTGAAGTCAC TTTTGACATT

1441 GATGCCAATG GTATACTCAA TGTCTCTGCT GTGGACAAGA GTACGGGAAA AGAGAACAAG

1501 ATTACTATCA CTAATGACAA GGGCCGTTTG AGCAAGGAAG ACATTGAACG TATGGTCCAG

1561 GAAGCTGAGA AGTACAAAGC TGAAGATGAG AAGCAGAGGG ACAAGGTGTC ATCCAAGAAT

1621 TCACTTGAGT CCTATGCCTT CAACATGAAA GCAACTGTTG AAGATGAGAA ACTTCAAGGC

1681 AAGATTAACG ATGAGGACAA ACAGAAGATT CTGGACAAGT GTAATGAAAT TATCAACTGG

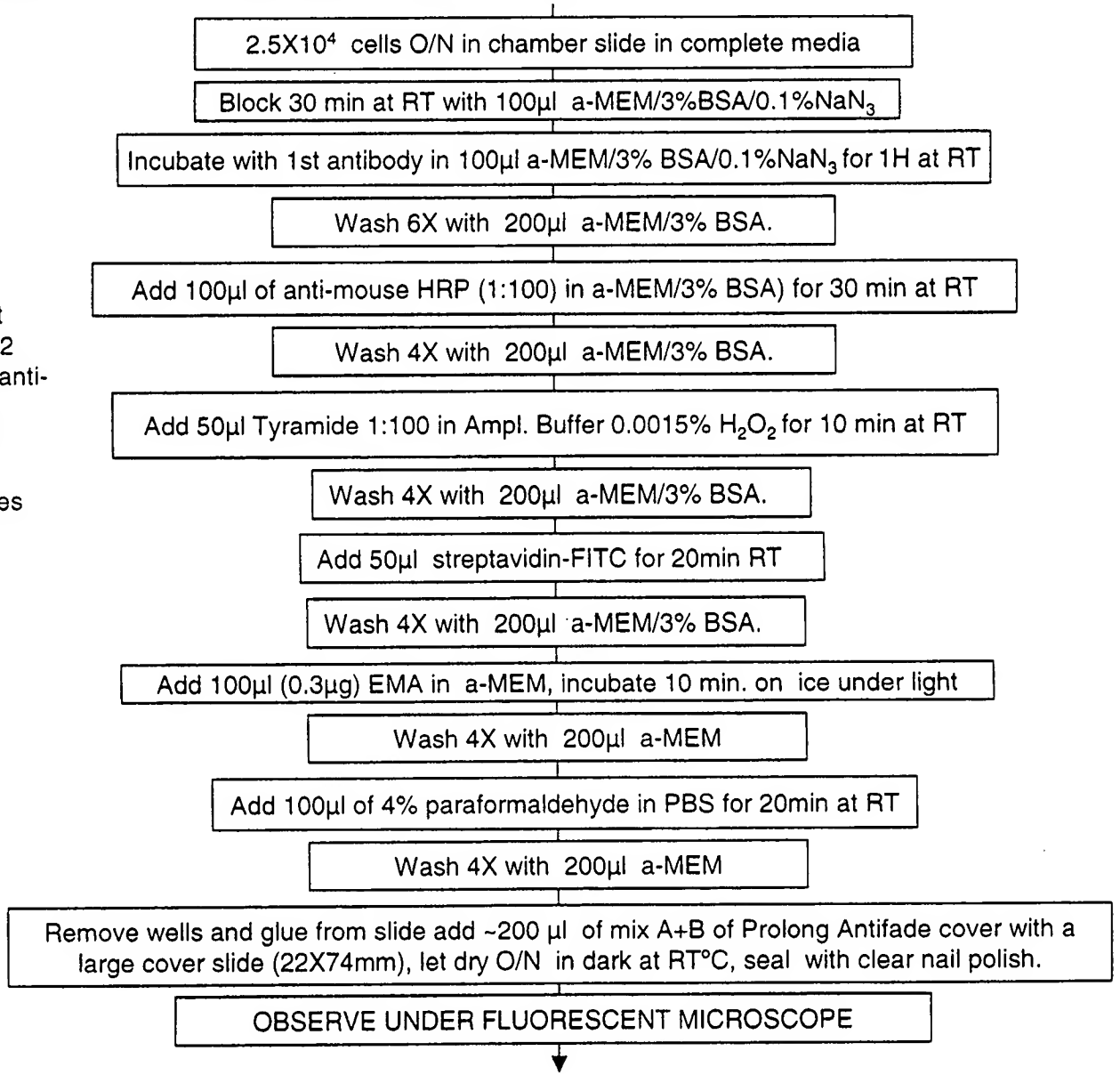
1741 CTTGATAAGA ATCAGACTGC TGAGAAGGAA GAATTTGAAC ATCAACAGAA AGAGCTGGAG

1801 AAAGTTTGCA ACCCCATCAT CACCAAGCTG TACCAGAGTG CAGGAGGCAT GCCAGGAGGA

1861 ATGCCTGGGG GATTTCCTGG TGGTGGAGCT CCTCCCTCTG GTGGTGCTTC CTCAGGGCCC

1921 ACCATTGAAG AGGTTGATTA A

Figure 15A : Procedure for immunofluorescence (non-permeabilized cells)



Amplification kit used: TSA kit #2 with HRP-goat anti-mouse IgG and Alexa fluor 488 tyramide from molecular probes T-20192

Figure 15B: Procedure for immunofluorescence (permeabilized cells)

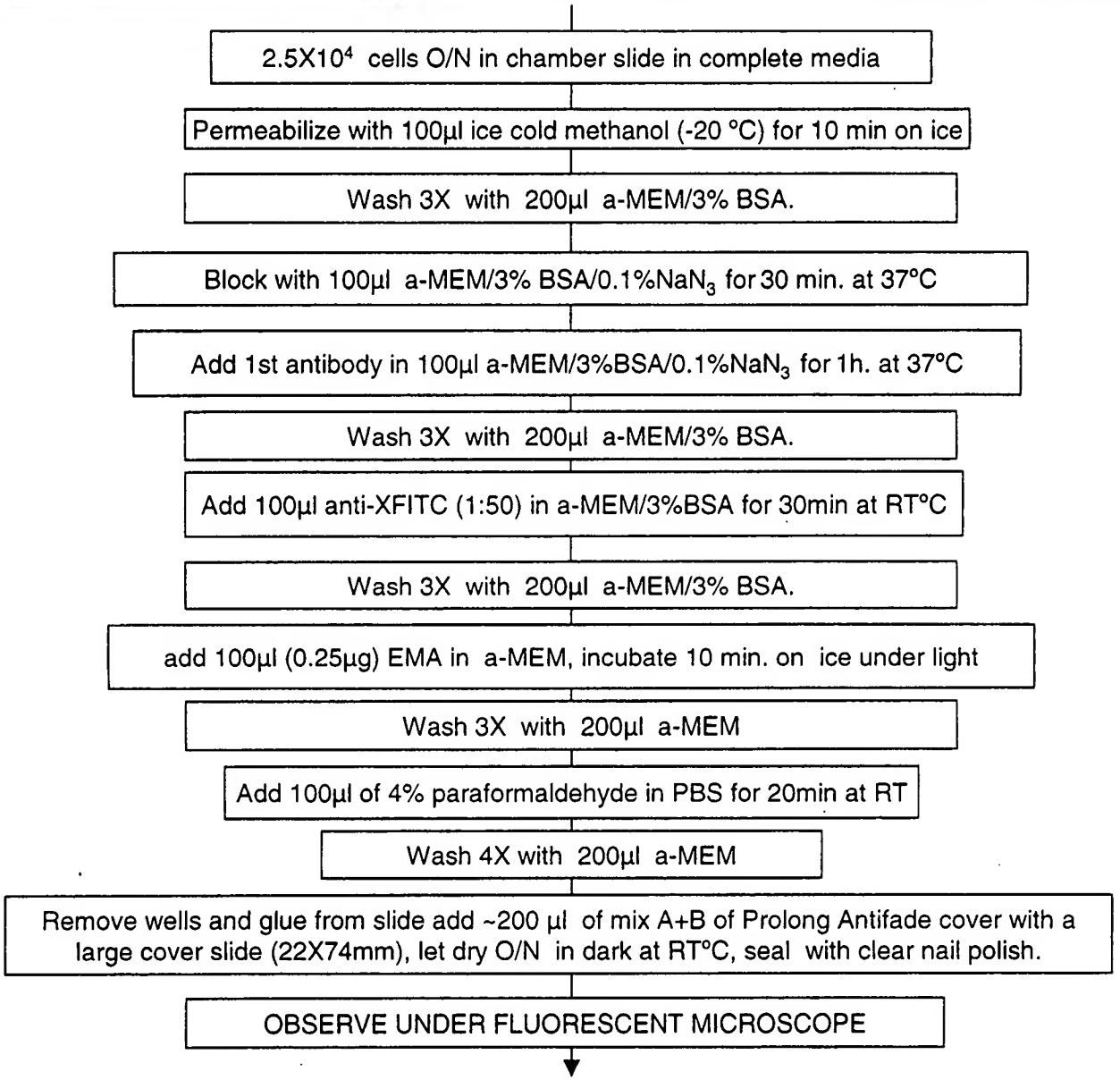


Figure 16: Immunofluorescence of surface exposed HSC70

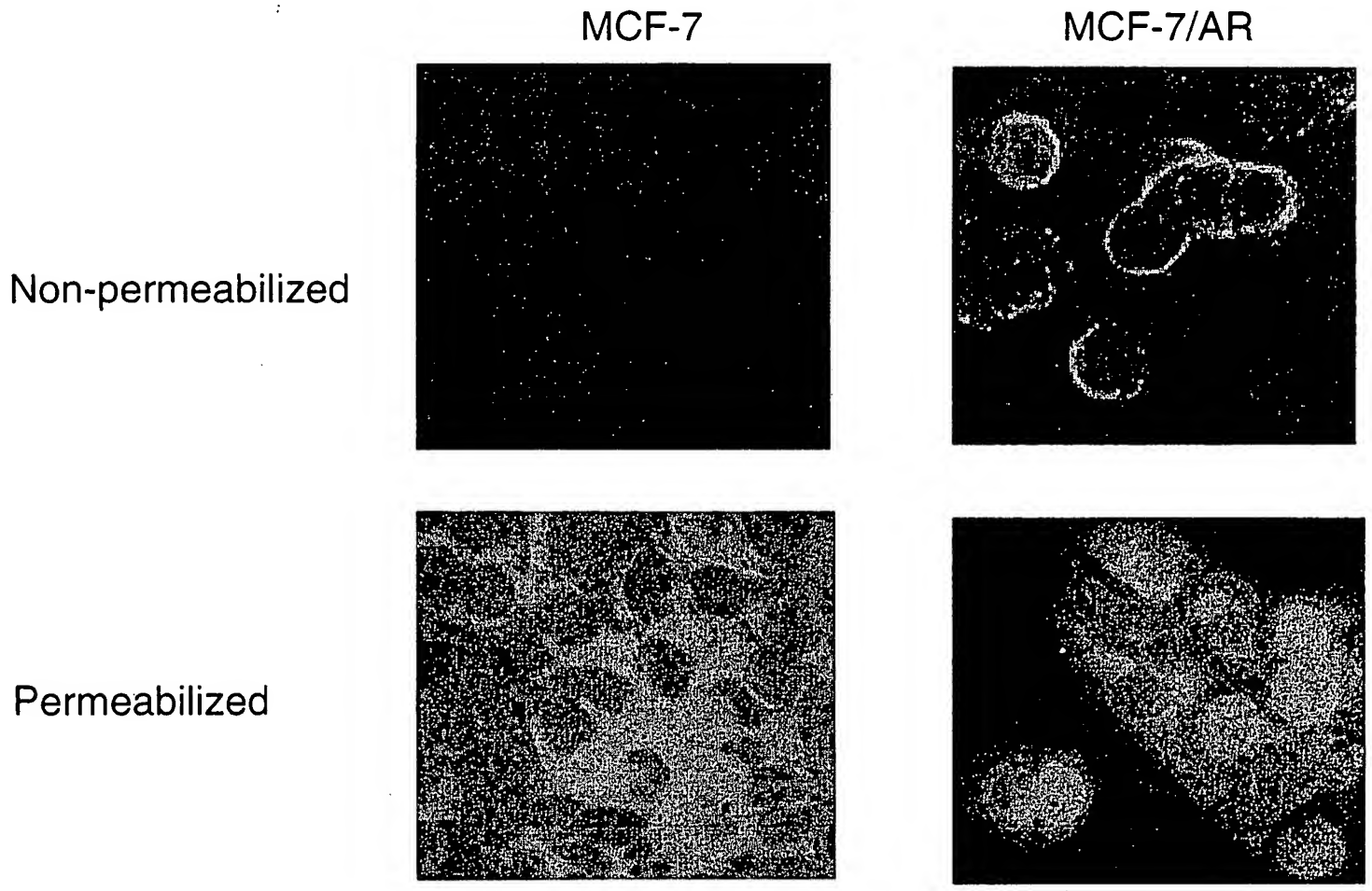


Figure 17: HSC70 expression

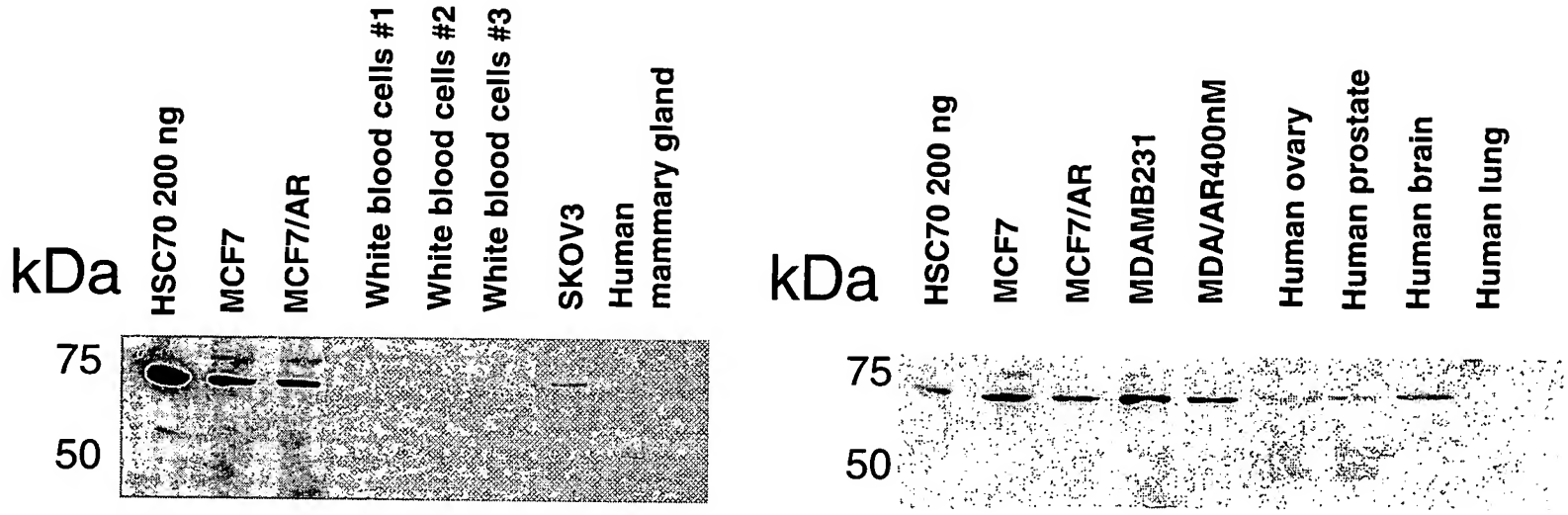


Figure 18